

436a

Kar3 may not undergo all of the structural transitions that the Kar3/Cik1 or the Kar3/Vik1 heterodimer do in vivo.

2400

#### Effects of hypoxia on spindle dynamics and a bidirectional spindle motor in live cells

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Early *Drosophila* embryos respond to low levels of oxygen by arresting development in interphase or metaphase. Although the response by *Drosophila* to oxygen deprivation is known to involve the nitric oxide pathway, the effects of hypoxia on cell cycle progression are not fully understood. We find that hypoxia has striking effects on centrosomal and spindle microtubules, indicating that it suppresses microtubule dynamics. Remarkably, a mutant of the minus-end Ncd spindle motor that moves towards either the microtubule plus or minus end in microtubule gliding assays *in vitro* shows no mutant effects in live oocytes or embryos. But oxygen-deprived mutant embryos show highly unusual effects on centrosomal and spindle microtubules that can be explained by plus-end movement of the mutant motor. We attribute these mutant effects under hypoxia but not normal conditions to suppression of plus-end motility under normal conditions by microtubule dynamics, uncovering an unexpected interaction between microtubule dynamics and Ncd motility in the cell.

2401

#### The *Arabidopsis* kinesin, KATA, is a minus-end directed kinesin involved in male meiosis

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The processes governing meiosis, specifically the formation and maintenance of the spindle apparatus, are not well defined in plants. Relatively few meiotic mutants that have defective meiotic spindles have been extensively characterized. We have isolated an *Arabidopsis* mutant with reduced male fertility and an abnormal meiotic spindle array. This mutant, *kata-1*, contains a transposon insertion in the KATA gene, which, based upon a homology to other kinesins in the motor domain, codes for a kinesin-like protein. During male meiosis the *kata-1* mutant has an atypical polar spindle with microtubules that are unfocused at the poles. As a result, chromosome segregation is abnormal and > 4 microspores are typically produced from each meiotic event. In the mutant, most microspores fail to develop into functional pollen, resulting in dramatically reduced pollen production and viability, and smaller siliques. Interestingly, there is no apparent defect in mitosis or in female gamete formation suggesting that KATA is only required in male meiosis, although it may be active in other cells. Bacterially expressed recombinant KATA shows motility *in vitro*, and moves at a velocity of 0.18 microns/sec. Motility assays using minus-end rhodamine labeled microtubules show that KATA moves towards the minus-end of microtubules. Furthermore, results from landing assays are consistent with a non-processive motor, however additional studies are in progress. Thus, KATA is a minus-end motor that participates in either meiotic-spindle formation or maintenance, and can be used to shed light on some of the processes involved in plant meiosis.

2402

#### Phosphorylation of Kid, kinesin-like DNA binding protein, regulates its chromosomal localization in M phase

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Microtubule-associated motor proteins are thought to be involved in spindle formation and chromosome movements in mitosis and meiosis. Kid is a member of kinesin family proteins, carrying a kinesin-like motor domain in the N-terminal half and a DNA binding domain near the C-terminus. Immunofluorescence studies showed that Kid localized with mitotic chromosomes or spindles, and that it was enriched in the kinetochore at anaphase. Cells overexpressing the DNA binding region of Kid showed abnormal phenotypes in chromosome condensation and spindle formation. These data indicate that Kid may play important roles for chromosome segregation.

To elucidate the function of Kid in metaphase, we analyzed the molecular mechanisms how the DNA binding activity of Kid was regulated. It is known that entry into mitosis is regulated by phosphorylation of many proteins. Using phosphopeptide mapping, we identified that Ser427, Thr463 and a few other amino acids of Kid were phosphorylated in metaphase. And we identified Thr463 as a Cdc2 kinase phosphorylation site. Gel shift assay showed that the highly phosphorylated Kid proteins in G2/M phase bound to target nucleotides. However, immunofluorescence studies with anti-phosphoThr463Kid antibodies showed that phosphorylated Kid at Thr463 did not localized along chromosome,

but along spindles. Gel shift assay also indicated that the non-phosphorylatable mutant (Thr463 to Ala mutant) Kid did not lose its DNA binding activity. These data suggest that indicated phosphorylation of Thr463 is not essential for binding to DNA directly, but it regulates chromosomal localization of Kid during mitosis. Taken together with our data, it is thought that phosphorylation of Thr463 is required for other KidOs functions, for example, motor activity or interaction to other molecules.

2403

#### Mechanism of the Small Molecule Eg5 Inhibitor Monastrol

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The kinesin Eg5 is important during organization of the mitotic spindle. The motor function of Eg5 is inhibited by the small molecule monastrol. We investigated the inhibitory mechanism as well as structure-activity relationship of monastrol using a monomeric Eg5 motor domain construct. During steady-state ATPase experiments, monastrol is not competitive with either ATP or microtubule binding. Equilibrium binding experiments demonstrate that monastrol weakens Eg5- nucleotide binding. The drug also interferes with Eg5-microtubule binding in the presence of ADP. Our experimental data suggests a mechanism for the inhibition of a particular step in the Eg5 catalytic cycle by monastrol.

2404

#### Two related kinesins, *klp5*<sup>+</sup> and *klp6*<sup>+</sup>, foster microtubule disassembly and are required for normal chromosome movement in fission yeast

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The kinesin super family of microtubule motor proteins is important in many cellular processes, including chromosome segregation and the establishment and maintenance of cell polarity. We have characterized two related kinesins in fission yeast, *klp5*<sup>+</sup> and *klp6*<sup>+</sup>, that are amino-terminal motors of the KIP3 subfamily. Analysis of null mutants demonstrates that neither *klp5*<sup>+</sup> nor *klp6*<sup>+</sup>, individually or together, is essential for vegetative growth, though these mutants have altered microtubule behavior and are essential for meiosis. *klp5Δ* and *klp6Δ* are resistant to high concentrations of the microtubule poison thiabendazole and have abnormally long cytoplasmic microtubules that can curl around the ends of the cell, suggesting Klp5/6p promote microtubule disassembly. This phenotype is greatly enhanced in the cell cycle mutant *cdc25-22*, leading to a bent cell morphology as enhanced in the cell cycle mutant *cdc25*<sup>+</sup> in microtubule behavior. The cells elongate, suggesting a role for *cdc25*<sup>+</sup> in microtubule behavior. The chromosomes in *klp5Δ* and *klp6Δ* null mutants do not show a normal metaphase alignment; chromosome pairs move back and forth along the spindle prior to sister chromatid separation. Ultimately, sister chromatids separate, regardless of chromosome position along the spindle, usually producing viable daughter cells. Anaphase B initiation is sometimes delayed, but elongation occurs at wild type rates, even as the chromosomes are still moving to the spindle poles. These null mutants are synthetically lethal with the spindle assembly checkpoint mutant *bub1Δ*, and several mutants of the anaphase promoting complex. Klp5p-GFP and Klp6p-GFP localize to interphase microtubules, and kinetochores from prophase to anaphase A, but they relocalize to the spindle midzone in anaphase B. Klp5p and Klp6p localizations are not co-dependent. These data suggest that Klp5p and Klp6p are kinetochore kinesin motors required for normal chromosome movement in prometaphase, perhaps through fostering microtubule disassembly.

2405

#### The role of the neck domain in the microtubule depolymerization activity of MCAK

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Mitotic centromere-associated kinesin (MCAK) (L. Wordeman and T. J. Mitchison, 1995, J Cell Biol, 128:95-104) is a member of the Kin I kinesin subfamily. Overexpression of full length MCAK in cells results in depolymerization of interphase and spindle microtubules (Maney et al. 1998, J Cell Biol, 142:787-801; Maney et al. 2001, JBC, in press). The conserved motor domain of MCAK (D246 - S583) alone is not able to depolymerize microtubules *in vivo* and *in vitro*. The addition of 64 amino acids of the MCAK neck domain (A182 - D246) to the amino terminus of the motor domain is necessary and sufficient to achieve depolymerization activity equal to (and perhaps better than) the activity of the full length MCAK (Maney et al. 2001, JBC, in press). To study the role of the neck domain in the depolymerization activity of MCAK, we generated a series of GFP-hamster-MCAK fusion constructs with mutations in the neck domain. By overexpressing the GFP-MCAK neck mutants in CHO cells, we were able to assay the microtubule depolymerization activity of these mutants. MCAK with a deletion of the whole neck domain was not capable of depolymerizing microtubules in cells although centromere-binding and nuclear localization was unaffected. MCAK mutants with smaller deletions or with specific alanine substitutions of highly conserved charged amino acids in the neck domain showed significant reduction of the microtubule depolymerization activity. These findings demonstrate the important role of the neck domain in the depolymerization activity of MCAK.